

HUMAN COMPLEMENT C4a DES ARG ASSAY LAYOUT SHEET
for use with Assay Designs' Catalog Nos. 900-059 and 901-059 (formerly 90059 and 90159)

- Invert the human C4a Conjugate 4-5 time immediately before use.
- DILUTION TABLE FOR MAKING STANDARDS 1-5:

Std.	Assay Buffer 10, 1x Vol. (µL)	Vol. Added (µL)	h Cmpl. C4a Conc. (ng/mL)
1	900	100, Stock	200
2	750	250, Std. 1	50
3	750	250, Std. 2	12.5
4	750	250, Std. 3	3.13
5	750	250, Std. 4	0.78

- ASSAY PROTOCOL FLOW CHART:

Well I.D.:	Blank A1, B1	TA C1, D1	NSB E1, F1	Bo G1, H1	Stds. A2 - B3	Samples C3-H12
Assay Buffer 10, 1x	---	---	150µL	100µL	---	---
Std. and/or Sample	---	---	---	---	100µL	100µL
Conjugate	---	---	50µL	50µL	50µL	50µL
Antibody	---	---	---	50µL	50µL	50µL
Incub. 2 hrs, shaking @ RT						
Asp. & Wash 3 x 200µL						
Conjugate	---	5µL *	---	---	---	---
Substrate	200µL	200µL	200µL	200µL	200µL	200µL
Incub. 1 hour @ 37°C, sealed						
Stop Solution	50µL	50µL	50µL	50µL	50µL	50µL

* Conjugate for TA must be diluted 1:10 in Assay Buffer 10. Add 500 µL of Assay Buffer 10 to a clean test tube. From this volume, remove 50 µL of Assay Buffer 10 and, using the same measuring device, add 50 µL of h C4a Conjugate. See Step 4, Reagent Preparation, on Page 5 of the enclosed kit insert.

HUMAN COMPLEMENT C4a DES ARG PLATE LAYOUT:

A1 Blank	A2 Std 1	A3 Std 5	A4	A5	A6	A7	A8	A9	A10	A11	A12
B1 Blank	B2 Std 1	B3 Std 5	B4	B5	B6	B7	B8	B9	B10	B11	B12
C1 TA	C2 Std 2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1 TA	D2 Std 2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E1 NSB	E2 Std 3	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F1 NSB	F2 Std 3	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G1 Bo	G2 Std 4	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H1 Bo	H2 Std 4	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

Kit Lot No. _____ Exp. Date _____ Date _____ Tech. _____

1st Incub.: Start Time _____ Temp. _____

End Time _____ Temp. _____

Notes: _____

2nd Incub.: Start Time _____ Temp. _____

End Time _____ Temp. _____
